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ORIGINAL ARTICLE

# Diagnostic and Prognostic Utility of FFAR2 Gene Expression in Peripheral Blood as a Potential Biomarker for Acute Coronary Syndrome

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## ABSTRACT

**Background:** Acute coronary syndrome (ACS) is the leading cause of morbidity and mortality. Presently, there is no convenient marker that can precisely expect the onset of ACS besides its prognosis. Free fatty acid receptor 2 (FFAR2) has lately been proposed to protect from heart diseases. The study aimed to assess whether the expression of FFAR2 in peripheral blood is avaluable biomarker for diagnosis of ACS and prediction of adverse cardiac events (ACEs) among ACS patients with controlled and uncontrolled DM.

**Methods:** Peripheral venous leukocytes were collected from 281 patients,133 as control group (Group I) and 148 diabetic patients admitted with ACS (Group II). GroupII was subdivided into Group II a, 67 ACS patients with controlled blood glucose and Group IIb, 61 ACS patients with uncontrolled blood glucose. The expression level of FFAR2 was estimated by real time PCR.

**Results:** FFAR2 expression in ACS patients was significantly lower than that in the control group. Moreover, the FFAR2 level in ACS with uncontrolled blood glucose was lowerthan that in ACS patients with controlled blood glucose level. The ROC curve analysis showedthat FFAR2 level had excellent sensitivity and moderate specificity to identify patients at riskof having ACE.

**Conclusions:** FFAR2 could act as a potential biomarker in diagnosis of ACS and predicting its complications.

**Keywords:** ACS; FFAR; ACE; Real time -PCR.

## INTRODUCTION:

Cardiovascular disease continues to be the leading cause of mortality worldwide, with ischemic heart disease accounting for approximately half of all deaths, despite major improvements in the detection and treatment of acute coronary syndrome (ACS) [1]. Consequently, there is an urgent needfor an efficient marker that can be used to predict the onset of ACS and its consequences with ease .The term "acute coronary syndrome" (ACS) is a range of clinical manifestations that develop after coronary plaque rupture and are exacerbated by varying degrees of thrombosis, embolization, and myocardial perfusion blockage. It includes three conditions that affect the coronary arteries: unstable angina (UA), non-ST segment elevation myocardial infarction (NSTEMI), and ST segment elevation myocardial infarction (STEMI) [2].

Free fatty acid receptors (FFARs) were initially identified as FFA receptors for dietary FFAs and related intestinal products of microbial fermentation in the digestive tract. Studies have shown that FFARs, which are nutrition sensors expressed in a variety of cells and tissues, also control immune responses and energy metabolism [3].

A group of typical intronless genes on chromosome 19q13.1 includes FFAR2, also identified as G-protein coupled receptors 43 (GPR43). It codes a member of the G protein-coupled receptor, GP40 family [4]. The first and second extracellular rings of the receptor produced by the FFAR2 gene have cysteine residues that can control conformation by creating intramolecular disulfide bonds [5]. FFAR2, which acts as a signaling molecule, is crucial in inflammation, regulation of serum lipid and blood sugar levels, and energy homeostasis throughout the body [6,7]. So, it is important to investigate the relationship between FFAR2, ACS, and T2DM.

A higher chance of developing ACS relates to

diabetes, which hastens the progression of atherosclerosis (ACS). Approximately 25-30% of this ACS patients admitted were diabetic. A diabetic patient is more likely to develop ACS, this is linked to an increased risk of death and more ischemia events. Poor prognosis in diabetic patients is related to increased proinflammatory and prothrombotic conditions [8]. Hyperglycemia has several detrimental biological effects in ACS patients. Augmented liberation of fatty acids and increased their blood levels, decreased glucose expenditure, amplified oxidative damage, compromised endothelial function as well as disturbed insulin secretion [9].

Patients with acute coronary syndrome ACS patients are more liable for emerging othermajor adverse cardiovascular events (MACEs). About 30% of ACS patients are type 2diabetics, and their risk of relapse of MACE is up to double that of non-type 2 diabetics. Other ischemic cardiovascular events are very likely to arise in patients with ACS. Type 2 diabetic patients have an approximately double risk [10].

**The aim of our work** was to investigate the role of FFAR2 gene expression from peripheral blood leukocytes as a molecular biomarker for diagnosis of ACS and as a potentialprognostic biomarker for ACEs among ACS patients with controlled and uncontrolled DM.

## METHODS

**Research Subjects:**

Between July 2022 and May 2023, 281 patients with chest pain indicative of ACS wereadmitted to the Cardiology Department and were involved in the study. It was carried out in the Medical Biochemistry Department and the Cardiology Department, Zagazig Faculty of Medicine. Subjects were divided into two categories: 133 patients in group I acted as the control group and were hospitalized due to complaints of pericardial pain. The absence of coronary artery abnormalities in those persons was further confirmed by cardiac catheterization. Thus, they were chosen as the control. 148 diabetic patients who had been hospitalized with ACS were in group II. It was split into group IIa, which consisted of 67 ACSpatients with controlled blood glucose whose HbA1c level is < 7%, and group IIb, which consisted of 61 ACS patients with uncontrolled blood sugar whose HbA1c level is >7%. We excluded patients with previous interventions which markedly affect FFAR2 expression level like PCI (percutaneous coronary intervention) and CABG (coronary artery bypass graft). The following criteria were used to determine which subjects were included in group I: patients who were hospitalized with chest discomfort but whose coronary angiography revealed no significant lesions. Group II (ACS) patients were verified by ECG changes and coronary angiography which show significant lesion. Informed consent was gained from all participants prior to the research. The research was applied in compliancewith Declaration of Helsinki. The study was approved ethically by the Zagazig University institute review board with approval number IRB#: 9853-3-7-2022 . The following was administered to each participant: ECG was performed to detect ischemic abnormalities after a thorough history taking that included age, sex, history of smoking, hypertension, and diabetes.We carefully examined and documented the levels of fasting blood sugar, HbA1c and lipid profile.

**Research Method**

**Collection of venous blood samples**

Peripheral venous blood samples were withdrawn on admission for real-time PCR investigation of plasma FFAR 2 levels. For diagnosing ischemia, coronary angiography was performed. Patients' adverse cardiac events were tracked to be correlated with FFAR2 level.

RNA extraction and synthesis of cDN

RNA extraction was performed using QIA amp RNA Blood Mini Kit Cat. no. 52304 from QIAGEN, Germany. All steps were performed in an environment free of RNAcontamination. Extraction was done on ice according to the instructions of the kit. Then, the extracted RNA was reverse transcribed and amplified using RT2 qPCR Primer Assay kit, Cat. no.330001 from QIAGEN, Germany. The cDNA was transferred to a –20°C freezer.

**Real time PCR analysis for FFAR2 expression**

The amplification was performed in a 25 µL mixture containing 5µL of the cDNA, 1 µL of RT² qPCR Primer Assay, 10 µL RT² SYBR® Green Mastermix and 10.5 µL RNase- free water. The PCR components mix were prepared in 5 ml tubes and placed in the real time cycler (Stratagene Mx3005P) qPCR System according to the following protocol: 95ºC for 10 min initial activation step then 40 cycles of 95ºC for 15 sec, 60ºC for 1 minute. GAPDH was used as internal reference gene and FFAR2 as target gene and their primer sequence is shownin Table 1. FFAR2 gene expression was calculated by $2^{-\Delta\Delta Ct}$ method.

## STATISTICAL ANALYSIS:

The clinical data were noted on a report. They were tabulated and analyzed using SPSSversion 20. The tests of significance between the different groups were tested. The ROC Curve was performed to measure cutoff value to recognize patients liable of ACE. Binary logistic regression was done to evaluate risk factors for ACE. The results were

statistically significantwith p value ≤ 0.05.

## RESULTS:

A total of 261 people participated in our study. 153 men (58.6%) and 108 women(41.4%) participated. The average age was 39.0-76.0 years old. Patients were divided into three groups: group I (non - coronary cardiac disease), group IIa (controlled blood sugar ACS patient), and group IIb (not controlled blood sugar ACS patients).

Demographic characteristics and risk factors in the three studied groups were shown in Table 2. There was non-significant difference regarding age and gender between groups. The Mean± SD for age was (55.11± 5.81) in group I and (54.24± 6.85) in groups IIa while in group IIb it was 56.54± 6.74. Regarding gender, group I consists of 66 males (49.62%) and 67 females (50.3%). Group IIa included 33 males (49.2%),34 females (50.7%), whereas group IIb included 31 males (50.8%) and 30 females (50.8%). The groups were comparable in terms of hypertension, smoking, and family history. There were not significantly differences among the three groups in terms of OHD (oral hypoglycemic drugs). Meanwhile, TC, TG, LDL-C, FBS and HbA1c were increasing steadily from group I to groups IIa and group IIb whereas HDL-C was decreasing steadily from group I to group IIa and group IIb as illustrated in (Table 2).

Regarding FFAR2 expression level, the results revealed that the relative expression Mean± SD of FFAR2 gene in group I was (1.02 ± 0.05) and (0.37± 0.06) in group IIa, while in group IIb, it was (0.19± 0.08) (Table 3, Figure1).

The FFAR2 expression was significantly lower in group IIa than in group I. The FFAR2 gene's relative expression was significantly lower in group IIb compared to groups I and IIa. It was also significantly lower in group IIa than in group I (Table 3, Figure 1).

Table (3) and figure (S1) presents adverse cardiac events (ACE) in the three groups. None of patients in group I, 19 (28.4%) patients in group IIa and 39 (63.9%) patients in group IIb were diagnosed having ACE with significant variance among the three groups (p<0.001).

Table (4) illustrates correlation between FFAR2 expression level and different parameters in the three groups. It was found that the study revealed significant positive correlation between FFAR2 level and HDL (r=0.556, p<0.001) in group I and with FBS (r=0.250, p=0.041) in group IIa. In group IIb, there were significant positive correlation between FFAR2 expression level with age (r=0.296, p=0.021) and HDL (r=0.417, p=0.001) the study revealed a significant negative association between FFAR2 expression level with FBS (r= -0.333, p=0.009).

Table (5) shows relation between ACE with FFAR2 expression level and laboratory data in the three groups. In group IIa, patients with ACE showed significant decline in FFAR2 expression level (p=0.003) and while they showed significant elevation in HbA1c (p=0.05) when compared to patients without ACE. In group IIb, patients with ACE showed significant increase in LDL-c (p=0.023), HbA1c (p=0.007) and total cholesterol (p=0.023) compared to patients without ACE while patients with ACE showed significant decrease in FFAR2 expression level (p=0.029) when compared to patients without ACE.

Binary logistic regression analysis showed that FFAR2 expression level (OR= 20.76, p= 0.037) and HbA1c (OR= 3.81, p= 0.013) were significant independent predictor of ACE as illustrated in Table (6)

The above significant factors in table 6 were put into multivariate logistic regression analysis to further evaluate significant predictors for ACE (Table 6). Factors significant in the univariate analysis were adjusted in the multivariate model.

ACE was significantly associated with LDL-C (OR = 17.6, p = 0.024) and HbA1c (OR = 3.812, p = 0.013). Meanwhile, it was found that FFAR2 expression level was a protective (decreased risk) factor for ACE.

Furthermore, the ROC curve analysis showed that FFAR2 expression level at cutoff.

0.4. had excellent sensitivity (100%) and moderate specificity (67.3%) to identify patients at risk of having ACE (AUC= 0.871, p<0.001) (Table S1, Figure S2).

**Table 1:** Primers of the studied gene:

| Genes | Genes primer sequence (5′–3′) |
|---|---|
| **FFAR2** | F CTTCGGACCTTACAACGTGTC |
| | R CTGAACACCACGCTATTGAC |
| **GAPDH** | F TGTGGGCATCAATGGATTTGG |
| | R ACACCATGTATTCCGGGTCAAT |

F: indicates forward. R: indicates reverse.

**Table 2:** Demographic characteristics & risk factors among the three studied groups

| | | Group I (n=133) | | Group IIa (n=67) | | Group IIb (n=61) | | Test value | p- value | p- value between groups | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | | | I Vs. IIa | I Vs. IIb | IIa Vs. IIb |
| **Age (years)** | **Mean± SD.** | 55.11± 5.81 | | 54.24± 6.85 | | 56.54± 6.74 | | 12 | 0.324 | 0.91 | 0.657 | 0.631 |
| **Gender** | **Male** | 66 | 49.62 % | 33 | 49.2 % | 31 | 50.8% | 15.4 | 0.123 | 0.653 | 0.234 | 0.228 |
| | **Female** | 67 | 50.3% | 34 | 50.7 % | 30 | 49.18% | | | | | |
| **Hypertension** | | 35 | 26.3% | 34 | 50.7 % | 32 | 52.5% | 17.57 | <0.001‡ | 0.001 | <0.001 | 0.846 |
| **Smoking** | | 63 | 47.4% | 44 | 65.7 % | 46 | 75.4% | 49.3 | <0.001‡ | <0.001 | <0.001 | 0.103 |
| **OHD** | | 24 | 18.2% | 18 | 26.9 % | 17 | 27.9% | 3.13 | 0.209‡ | 0.156 | 0.126 | 0.899 |
| **Family history** | | 27 | 20.3% | 25 | 37.3 % | 37 | 60.7% | 30.7 | <0.001‡ | 0.010 | <0.001 | 0.008 |
| **TC (mmol/l)** | **Mean± SD. Range** | 4.56± 0.13<br>3.8- 5.3 | | 5.60± 0.39<br>3.9- 5.8 | | 6.36± 0.07<br>6.2- 6.5 | | 217.4 | <0.001ǂ | <0.001 | <0.001 | <0.001 |
| **TG (mmol/l)** | **Mean± SD. Range** | 1.33± 0.13<br>1.0- 1.96 | | 1.80± 0.04<br>1.60- 1.90 | | 2.40± 0.03<br>2.2- 2.5 | | 224.9 | <0.001ǂ | <0.001 | <0.001 | <0.001 |
| **LDL-C (mmol/l)** | **Mean± SD. Range** | 2.93± 0.13<br>2.30- 3.60 | | 3.78± 0.11<br>3.10- 3.90 | | 4.69± 0.06<br>4.30- 4.80 | | 233.5 | <0.001ǂ | <0.001 | <0.001 | <0.001 |
| **HDL-C (mmol/l)** | **Mean± SD. Range** | 1.59± 0.09<br>1.5- 1.7 | | 0.92± 0.07<br>0.8- 1.2 | | 0.48± 0.05<br>0.44- 0.75 | | 223.9 | <0.001ǂ | <0.001 | <0.001 | <0.001 |
| **FBS (mg/dl)** | **Mean± SD. Range** | 94.71± 12.60<br>70.0- 110.0 | | 113.21± 6.70<br>102.0- 125.0 | | 182.64± 22.41<br>130.0- 200.0 | | 193.2 | <0.001ǂ | <0.001 | <0.001 | <0.001 |
| **Hb A1c** | **Mean± SD. Range** | 5.19± 0.29<br>4.70- 5.70 | | 6.47± 0.35<br>6.0- 7.0 | | 8.62± 0.37<br>8.0- 9.5 | | 218.9 | <0.001ǂ | <0.001 | <0.001 | <0.001 |

**Table 3:** Comparison between the three studied groups regarding FFAR2 expression level and ACE:

| | | Group I (n=133) | Group IIa (n=67) | Group IIb (n=61) | Test value | p- value | p- value between groups | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I Vs. IIa | I Vs. IIb | IIa Vs. IIb |
| **FFAR2** | **Mean± SD.** | 1.02± 0.05 | 0.37± 0.06 | 0.19± 0.08 | **215.1** | **<0.001ǂ** | **<0.001** | **<0.001** | **<0.001** |
| **ACE** | **Negative Number** | 133<br>100% | 48<br>71.6% | 22<br>36.1% | **100.3** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| | **Positive Number** | 0<br>0% | 19<br>28.4% | 39<br>63.9% | | | | | |

ǂ Kruskal Wallis Test,

**Table 4:** Correlation between FFAR2 expression level and different parameters in the three groups.

| | Group I (n=133) | | Group IIa (n=67) | | Group IIb (n=61) | |
|---|---|---|---|---|---|---|
| | r | p- value | r | p- value | r | p- value |
| **Age (years)** | .006 | .943 | .228 | .064 | .296 | **.987** |
| **TC (mmol/l)** | -.004- | .961 | .163 | .189 | -.089- | .495 |
| **TG (mmol/l)** | -.093- | .287 | .092 | .460 | .104 | .425 |
| **LDL-C (mmol/l)** | -.071- | .419 | -.062- | .619 | .250 | .052 |
| **HDL-C (mmol/l)** | .556 | **.000** | .007 | .955 | .417 | **.001** |
| **FBS (mg/dl)** | .031 | .722 | .250 | **.041** | -.333- | **.009** |
| **HA1C** | .008 | .926 | .039 | .755 | .027 | .835 |

*r: Spearman rho*

**Table 5:** Relation between ACE with FFAR2 expression level and laboratory data in the three groups.

| | Group I (n=133) | | Group IIa (n=67) | | | | | Group IIb (n=61) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Negative | | Negative | | positive | | p- value♦ | Negative | | positive | | p- value♦ |
| | Mean | SD | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | |
| **FFAR2 expression level** | .97 | .25 | .39 | .04 | .33 | .09 | **.003** | .22 | .10 | .17 | .06 | **.029** |
| **TC (mmol/l)** | 4.56 | .13 | 5.63 | .34 | 5.51 | .50 | .271 | 6.35 | .08 | 6.39 | .04 | **.023** |
| **TG (mmol/l)** | 1.33 | .13 | 1.80 | .05 | 1.80 | .00 | 1.00 | 2.39 | .05 | 2.40 | .00 | .434 |
| **LDL-C (mmol/l)** | 2.93 | .13 | 3.77 | .13 | 3.80 | .00 | .520 | 4.67 | .09 | 4.70 | .00 | **.023** |
| **HDL-C (mmol/l)** | 1.59 | .09 | .92 | .07 | .91 | .08 | .137 | .48 | .03 | .48 | .05 | .917 |
| **FBS (mg/dl)** | 94.61 | 12.60 | 112.83 | 6.97 | 114.16 | 6.03 | .486 | 183.59 | 23.64 | 182.10 | 21.99 | .577 |
| **HA1C** | 5.20 | .29 | 6.42 | .33 | 6.59 | .36 | **.050** | 8.46 | .40 | 8.71 | .33 | **.007** |

♦ *Mann-Whitney U Test*

**Table 6:** Logistic regression analyses of independent risk factors for ACE

| Parameters | B | S.E. | Wald | P-value | Odds ratio (OR) | 95% CI | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower limit | Upper limit |
| **FFAR2 expression level** | -9.773- | 3.381 | 8.353 | **.004** | .0001 | .000 | .043 |
| **TC (mmol/l)** | -.638- | .654 | .953 | .329 | .528 | .147 | 1.902 |
| **LDL-C(mmol/l)** | 2.866 | 1.267 | 5.119 | **.024** | 17.566 | 1.467 | 210.331 |
| **HA1C** | 1.338 | .539 | 6.171 | **.013** | 3.812 | 1.326 | 10.956 |
| **Older age** | -.040- | .441 | .008 | .927 | .960 | .405 | 2.278 |

B: Regression coefficient; S.E.: Standard error, CI: Confidence interval

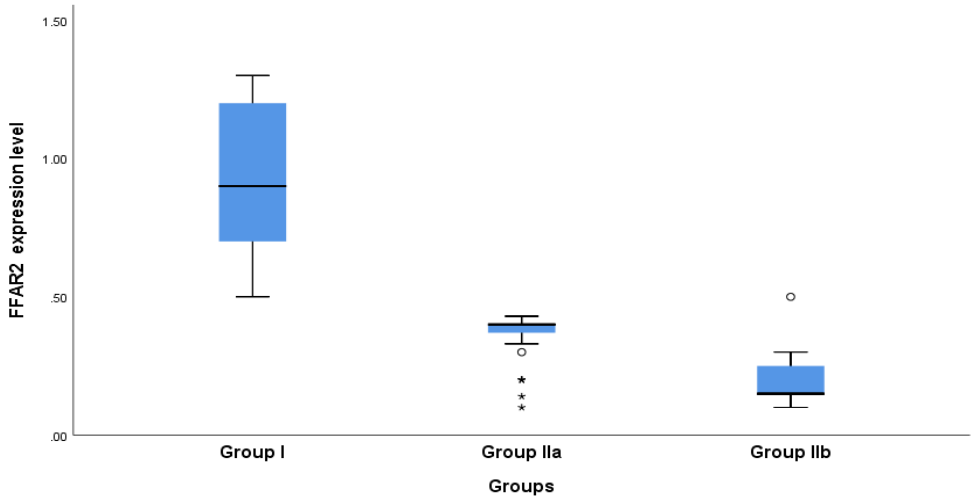


**Figure 1:** Relative expression of the FFAR2 gene in the three groups.

## DISCUSSION:

Despite major advancements in the identification and treatment of ACS, cardiovascular disease remains the principal mortality cause worldwide [11]. Finding molecular markers for quick identification is essential in informing physicians about the possibility of ACS.

Coronary heart disease (CHD) and diabetes mellitus (DM) are considered dual clinical conditions which strongly interact and become more common associated with each other [12].Our study revealed intergroup significant difference in total cholesterol, triglycerides,

LDL, HDL, fasting blood glucose and HA1c (Table 2).

Evolving evidence proposes that, in addition to DM, it has permanently been thought to have a metabolic substrate [13]. The risk of evolving and progressing coronary artery diseaseis also affected by metabolic abnormalities [14]. Diabetics experience all symptoms of coronary heart disease, including myocardial infarction (MI), as a minimum twice as often as those without diabetes. The current research showed that FFAR2 expression level in blood was steadilydecreasing from group I (control group) to group IIa (ACS patients with controlled blood glucose) and finally group IIb (ACS patients with uncontrolled blood glucose) showed the lowest FFAR2 level. This signifies that FFAR2 act as a protective factor against atherosclerosis and ACS. This may be attributed to its role in decreasing the production of proinflammatory cytokines that has a crucial role in atherosclerosis and ACS [19]. This result is with Ruan et al., who proved that FFAR2 level in AMI patients' peripheral blood was considerably lesser than that of control group. Little FFAR2 expression in peripheral blood is considered a self- ruling risk

75% of diabetes deaths are due to coronary heart disease [15].

The negative influence of diabetes on myocardial tissue and coronary circulation is caused by several pathophysiological mechanisms. These mechanisms involve changes in sodium manipulation and resultant circulatory overload, liberation of proinflammatory cytokines, accumulation of glycated end products and abnormal calcium manipulation [16,17]. Endothelial dysfunction is due to all of these pathways.

FFAR2 and other free fatty acid receptors control many vital processes, for instance adipogenesis, appetite control, intestinal movement, inflammation, carbohydrate expenditure and CNS function. Their expression in different tissues adds to its role in a number of human diseases, including obesity, gout, diabetes, arthritis, asthma and colitis, as well, their expression is reported to be changed in cardiovascular diseases such as hypertension, atherosclerosis and myocardial infarction [18].

determinant for AMI [19].

In the present study, we detected a considerable negative correlation between FFAR2 level and fasting blood glucose, which can be explained by its role in glycemic homeostasis [20]. Moreover, FFAR2 was shown to manipulate via the Gαq subunit, resulting in increasedintracellular calcium levels [Ca2+] and triggers the MAPK (mitogen-activated protein kinase)cascade [21]. The FFAR2-activated Gαq pathway has been proved to facilitate GLP1 secretionfrom intestinal L cells [22]. Thus, FFAR2 activation adds to stimulation of beta cell of Langerhans promoting insulin release to normalize blood glucose concentration [20]. FFAR2 stimulators could act as insulin sensitizer

enhancing their potential in treating of diabetic patients [23].

Sodium butyrate is a microbial metabolic product which could increase the FFAR2 level significantly, enhance glycogen stores, and keep glycemic regulation. Sodium butyrate can act via FFAR2-Akt-Gsk3 pathway. FFAR2 preserves fasting blood glucose via the FA signaling pathway [24]. FFAR2 is implicated in enhancing intestinal hormone release, decreasing fat breakdown, and modifying immune mediators. These valuable effects may be due to the indirect motivation of islet β-cells stimulating insulin release [7,25].

A positive considerable correlation between FFAR2 level and HDL has been proved in this research. This result is with Ruan et al [19] and with Ge et al., who demonstrated that activation of FFAR2 by acetate causes decline in plasma free fatty acid concentrations and anincrease in HDL-C levels and their results propose a possible impact for FFAR2 in modifyingplasma lipid levels [26]. Numerous research has demonstrated that HDL-C possesses a defensive anti-atherosclerotic effect unrelatedly of gender or race, mostly through its action as cholesterol scavenger from different tissues. Furthermore, HDL-C can impede thrombus formation by its anti-inflammatory and antioxidant effects [27].

FFAR2 triggering inhibit lipolysis via inhibition of the Gαi-facilitated cAMP/PKA path[28]. Thus, it is a major function of FFAR2 is to adjust adipose tissue energy buildup and adipogenesis, thus owning an important effect in the pathogenesis of the metabolic syndrome [29]. Propionate and acetate enhance FFAR2 in murine adipose tissue, resulting in diminishedplasma FFA concentrations and reduced lipolysis [30,31].

It was suggested that FFAR2 is an important intermediary in obesity caused by high fat diet (HFD) [32]. Conversely, further studies showed no role of FFAR2 on fatty tissue synthesisin vivo or in vitro, disproving the previous correlation between FFAR and fatness [32,33]. If upcoming research demonstrates a crucial function for FFAR in fat tissue regulation, its suppression via drugs could be helpful for cardiac patients [33].

FFAR2 has a crucial role in fatty cell distinction as well as its development.

Furthermore, propionate and acetate enhance lipid build up and inhibit lipolysis [30].

FFAR2 decreases producing of inflammatory intermediaries by repressing the synthesis of

Binary logistic regression analysis showed that low expression of the FFAR2 increasedthe hazard of ACE to 20.76 times (OR= 20.76, p= 0.037) and HbA1c (OR= 3.81, p= 0.013) were significant independent predictor of ACE.

cytokines. As well, it is involved in adjusting neutrophil triggering and influence their movement. Moreover, FFAR2 decreases the cytokines' synthesis via activating proinflammatory transcription factor (NF)-κB in heterologous cells [35]. Consequently, FFAR2 possesses a vital role in atherosclerotic inflammatory cascade.

Our results show relation between ACE with FFAR2 expression level and laboratory data in the three groups. In group IIa patients with ACE showed significant decline in FFAR2 expression level (p=0.003). Meanwhile, they showed significant elevation in HbA1c (p=0.05) when compared to patients without ACE. In group IIb, patients with ACE showed significant increase in LDL-c (p=0.023), HbA1c (p=0.007) and total cholesterol (p=0.023) compared to patients without ACE while patients with ACE showed significant decrease in FFAR2 expression level (p=0.029) when compared to patients without ACE.

Regarding cardiac adverse events, there were high significant variation between control group and ACS patients with controlled and uncontrolled diabetes mellitus. These findings arein accordance with Bjarnason et al. who demonstrated that ACS patients and Type 2 Diabetic patients had an augmented hazard of mortality, myocardial infarction and MACE whencontrasted to non-diabetics after follow-up [36]. A well-known hazard determinant for coronaryheart disease is high blood sugar levels. DM not only rises CAD development but is also associated with a greater level of CAD complexity [37]. Many studies have demonstrated thattype 2 diabetic patients presented by ACS have poorer outcomes than their non-diabetic counterparts [38].

Earlier studies of hyperglycemia CHD cases have steadily proved an augmented hazardof impending cardiac complications, in prediabetics during case pursue [39,40].

FFAR2 induces the synthesis of glucagon-like peptide (GLP)-1 [41]. This induction was approved in a previous study, performed on mice, and was believed to handle through the Gi inhibitory mechanism in enterocytes [42]. Although GLP-1 was revealed to own some protective cardiac functions, the way of its modulating by FFAR2 is entirely unidentified [43,44]. On the contrary, the FFAR2's special influence on GLP-1 highlight its potential importance in treatment of obesity and cardiac problems.

Factors significant in the univariate analysis were adjusted in the multivariate model. ACE was significantly associated with LDL-C (OR = 17.6, p = 0.024) and HbA1c (OR = 3.812, p = 0.013). Meanwhile, it showed that FFAR2 expression level

was a protective (decreased risk) factor for ACE. Furthermore, the ROC curve analysis showed that FFAR2 expression level at cutoff 0.4had excellent sensitivity (100%) and moderate specificity (67.3%) to identify patients at risk of having ACE (AUC= 0.871, p<0.001).

## CONCLUSIONS:

FFAR2 gene expression in the peripheral blood of ACS patients was lower than that inthe control group. The ROC curve analysis showed that FFAR2 expression level at cutoff 0.4 had excellent sensitivity (100%) and moderate specificity (67.3%) to identify ACS patients atrisk of having ACE. FFAR2 expression level is a protective (decreased risk) factor for ACE. Itis concluded that FFAR2 could act as a potential biomarker in diagnosis of ACS and predictingits complications (ACE). This will be very helpful for cardiologists to pick up patients requiring close observation and monitoring post ACS, hoping to decrease morbidity and mortality.

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**Table S1:** Validity of FFAR2 expression level in prediction of ACE**.**

| *Parameters* | *Best Cutoff value* | *AUC* | *Sensitivity* | *Specificity* | *PPV* | *NPV* | *P value* |
|---|---|---|---|---|---|---|---|
| **FFAR2 expression level** | ≤0.4 | 0.871 | 100% | 67.3% | 75.4% | 100% | <0.001 |

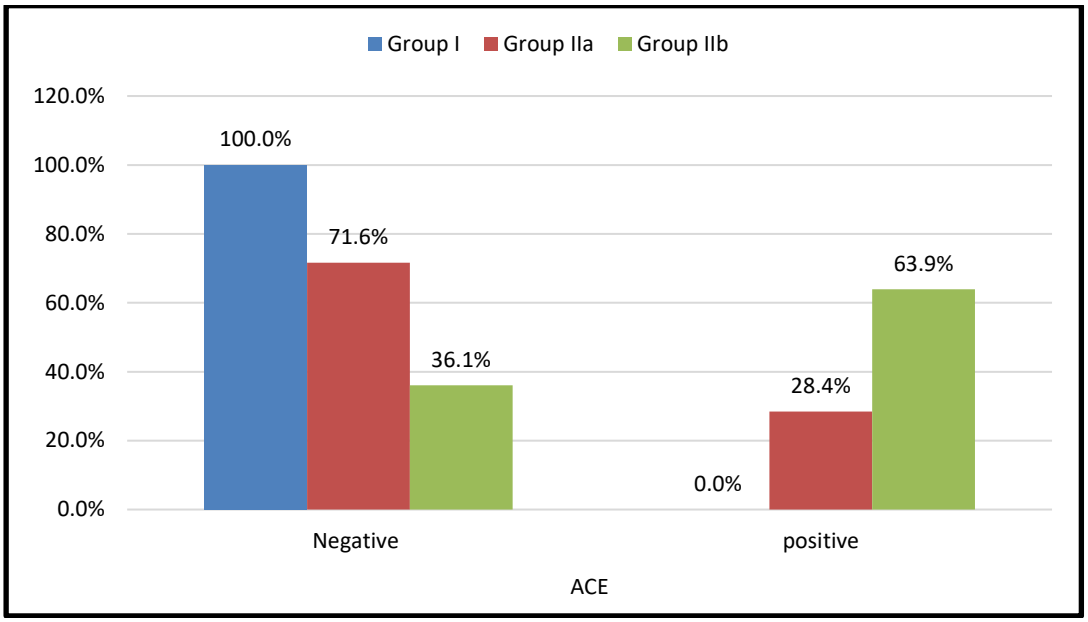


**Figure S1:** ACE (Adverse cardiac events) percentage among the three studied groups.

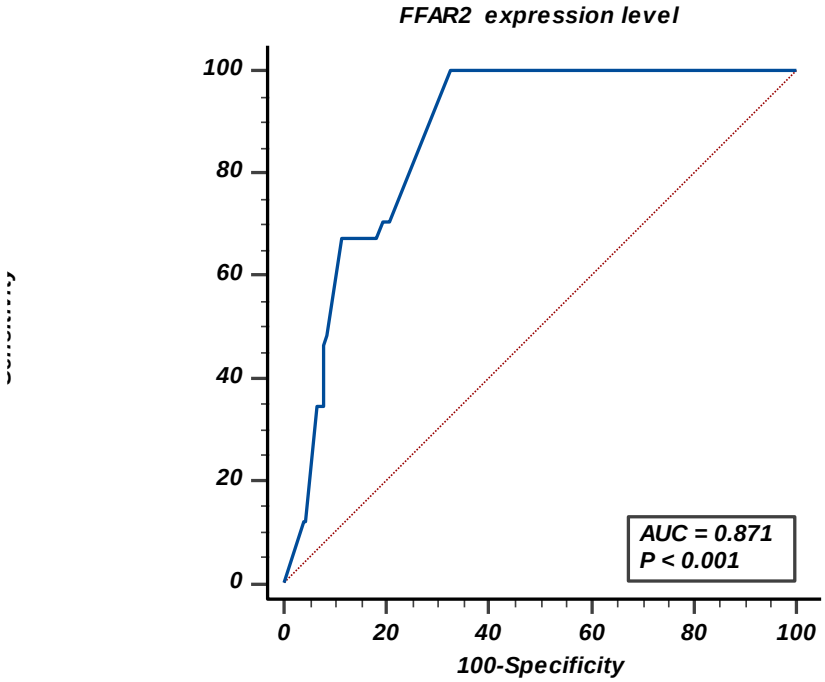


**Figure S2:** ROC curve for FFAR2 expression level in prediction of A